GUN BENGTSSON

# LACTATE DEHYDROGENASE IN MAMMALIAN TISSUES AND BODY FLUIDS AS RELATED TO ORGAN DEVELOPMENT AND TUMOUR GROWTH

LUND 1980

# LACTATE DEHYDROGENASE
# IN MAMMALIAN TISSUES AND BODY FLUIDS
# AS RELATED TO ORGAN DEVELOPMENT AND TUMOUR GROWTH

av

GUN BENGTSSON
Fil.mag., Ld

AKADEMISK AVHANDLING

som med vederbörligt tillstånd av Matematisk-Naturvetenskapliga Fakulteten vid Lunds Universitet för avläggande av filosofie doktorsexamen kommer att offentligen försvaras i Zoofysiologiska institutionens föreläsningssal, Lund, fredagen den 26 september 1980 kl 10.00.

Organization
LUND UNIVERSITY
Department of Zoophysiology
Helgonavägen 3B, S-223 62 Lund
Sweden

Document name
DOCTORAL DISSERTATION
Date of issue
September 1980
CODEN: LUNBDS/(NBZF-1007)1-24/(1980)

Author(s)
Gun Bengtsson

Sponsoring organization

Title and subtitle
Lactate dehydrogenase in mammalian tissues and body fluids as related to organ development and tumour growth.

Abstract
Total activity and isozyme pattern of lactate dehydrogenase (LDH) and malate dehydrogenase (MDH) in heart, liver , kidney, skeletal muscle, brain and serum were investigated during the ontogeny of gerbil, rat, mouse and rabbit. The total activity of LDH and MDH was measured spectrophotometrically and their isozymes were separated by gel electrophoresis. These parameters were found to change during organ development and differences between the species were revealed. The gerbil liver exhibited only one LDH isozyme, LDH 5, throughout the ontogeny.

The LDH activity in heart, liver, skeletal muscle and brain of mice bearing Ehrlich ascites tumour was investigated during tumour growth. A significant increase in liver LDH activity and a marked decrease in muscle LDH activity were observed. In serum and cell free ascites fluid of the tumour bearing mice the LDH activity increased during tumour growth. The elevation of the LDH activity of the ascites fluid was higher than that expected due to tumour cell death and the results of the study indicated a leakage of LDH from viable cells.

Also gerbils bearing Ehrlich ascites tumour exhibited elevated LDH activity in serum and cell free ascites fluid. The increase was transient, and the appearance and disappearance of tumour derived LDH in these body fluids were correlated to tumour cell death and tumour regression.

In rats and mice subjected to partial hepatectomy increased plasma LDH activities were observed 6 h after the operation. At 24 h the elevation still persisted in mouse plasma, while the LDH activity of rat plasma had returned to a normal level.

Key words
LDH, MDH, gerbil, rat, mouse, rabbit, ontogenetic development, liver, skeletal muscle, serum, Ehrlich ascites tumour, partial hepatectomy, heart, kidney, brain

Classification system and/or index terms (if any)

Supplementary bibliographical information

Language
English

ISSN and key title

ISBN

Recipient's notes

Number of pages
24

Price

Security classification

DOKUMENTDATABLAD enl SIS 61 41 21

Distribution by (name and address) Gun Bengtsson, University of Lund, Department of Zoophysiology, Helgonavägen 3B, S-223 62 Lund, Sweden

I, the undersigned, being the copyright owner of the abstract of the above-mentioned dissertation, hereby grant to all reference sources permission to publish and disseminate the abstract of the above-mentioned dissertation.

Signature Gun Bengtsson Date August 15, 1980

From the Department of Zoophysiology, University of Lund,

Lund, Sweden

---

# LACTATE DEHYDROGENASE
# IN MAMMALIAN TISSUES AND BODY FLUIDS
# AS RELATED TO ORGAN DEVELOPMENT AND TUMOUR GROWTH

by

GUN BENGTSSON

---

Lund 1980

This thesis is based on the following papers:

I. Bengtsson G.B. and Karlsson B.W. (1976) Lactate and malate dehydrogenase activities during the ontogeny of the mongolian gerbil, rat, mouse, and rabbit. Comp. Biochem. Physiol. 54B, 501-507.

II. Bengtsson G.B. and Karlsson B.W. Ontogenetic changes in lactate dehydrogenase and malate dehydrogenase isozyme patterns of the mongolian gerbil as compared with the rat, mouse and rabbit. Submitted for publication.

III. Bengtsson G. and Andersson G. The effect of Ehrlich ascites tumour growth on lactate dehydrogenase activities in tissues and physiological fluids of tumour bearing mice. Int. J. Biochem. (in press).

IV. Andersson G., Bengtsson G., Albinsson A., Rosén S., and Heby O. (1978) Increased polyamine concentration and tumor-derived lactate dehydrogenase activity in physiological fluids during regression of a heterologous tumor transplant. Cancer Res. 38, 3938-3943.

V. Yngner T., Bengtsson G., Carlberg E., Engelbrecht C., and Wieslander A. Possible significance of changes in the energy metabolism for the release of liver lactate dehydrogenase and for the uptake and incorporation of [$^{14}$C]-orotic acid into liver ribonucleic acid after partial hepatectomy. Expl. Cell Biol. (in press).

These papers will be referred to by their Roman numerals.

CONTENTS


| | Page |
|---|---|
| INTRODUCTION | 5 |
| TOTAL ACTIVITY AND ISOZYME PATTERN OF LDH IN VARIOUS ORGANS (I, II, III) | 7 |
| Liver | 8 |
| Skeletal muscle | 11 |
| Heart | 12 |
| Kidney | 13 |
| Brain | 13 |
| LDH ACTIVITY IN PHYSIOLOGICAL FLUIDS (I, III, IV, V) | 14 |
| Effect of tumour growth | 14 |
| Effect of partial hepatectomy | 16 |
| SUMMARY | 19 |
| ACKNOWLEDGEMENTS | 20 |
| REFERENCES | 21 |

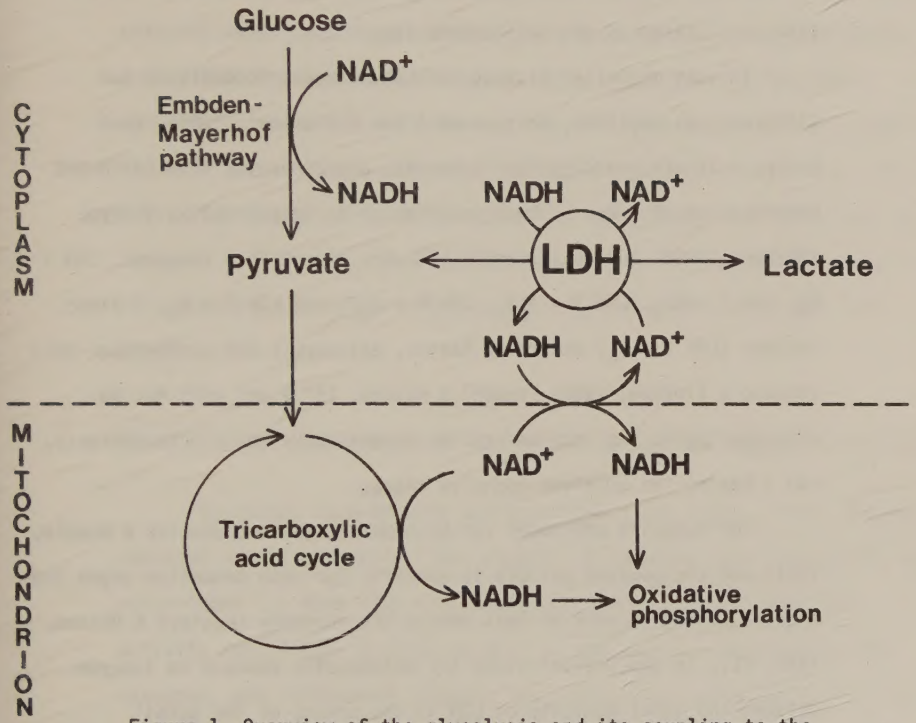


Figure 1. Overview of the glycolysis and its coupling to the tricarboxylic acid cycle and the oxidative phosphorylation.

The final step in glycolysis in the mammalian cell is the conversion of pyruvate to lactate (Fig. 1). This reaction is catalyzed by the enzyme lactate dehydrogenase (LDH, EC 1.1.1.27) in cooperation with the co-enzyme nicotinamide adenine dinucleotide (NAD). Under anaerobic conditions the reaction is important for the energy supply of the cell. The NADH produced in the Embden-Mayerhof pathway is oxidized during the conversion of pyruvate to lactate, and the $NAD^+$ formed allows the glycolysis to continue, yielding adenosine

triphosphate (ATP). This ATP production is needed by the cell when the oxidative phosphorylation does not work due to oxygen deficiency. With the appropriate oxygen supply the reaction is reversed and lactate is utilized in the cell metabolism.

In most mammalian tissues the LDH molecule is built by two different polypeptides, designated A and B (Markert, 1968). Each enzyme molecule contains four subunits, which permits five different combinations of A and B. Each combination is designated an isozyme (Markert, 1968) and consequently LDH consists of five isozymes, LDH 1 = $B_4$, LDH 2 = $AB_3$, LDH 3 = $A_2B_2$, LDH 4 = $A_3B$, and LDH 5 = $A_4$. A sixth isozyme (LDH X = $C_4$) occurs in testes, epididymis and spermatozoa only (Blanco & Zinkham, 1963; Lindahl & Mayeda, 1972) and will not be discussed here. The isozymes can be separated by gel electrophoresis, LDH 1 having the greatest negative charge.

The subunits are coded for by separate genes (McKusick & Ruddle, 1977) and the isozyme pattern is specific for each mammalian organ and species, changing more or less during the ontogeny (Masters & Holmes, 1975; II). In the present study the ontogenetic changes in isozyme pattern and total activity of LDH in the organs of the gerbil (Meriones unguiculatus) have been investigated. The gerbil is a new laboratory animal, which is gaining increasing use in different fields of research (Schwentker, 1975). Comparisons have been made with the more established laboratory animals rat, mouse and rabbit (I, II).

LDH also appears extracellularly. Normally the activity is low in the body fluids, but under certain circumstances it increases. It is known that pathological conditions which induce cell death cause an elevated LDH activity in serum (Latner & Skillen, 1968; Wolfe & Williams, 1973). In agreement with this, immunologically induced tumour cell death causes increased LDH activity in body fluids of the tumour bearing gerbil (IV). The results of the present study also indicate that viable

cells with an impaired membrane function might leak LDH to the extracellular fluids (III, V).

The present study focusses upon LDH changes chiefly in the liver, skeletal muscle and heart of developing gerbil, rat, mouse and rabbit and of mice bearing Ehrlich ascites tumour, and upon changed LDH activity in serum and cell free ascites fluid of tumour bearing gerbils and mice and in plasma of partially hepatectomized rats and mice. The biological significance of intracellular and extracellular LDH and its isozymes is discussed. For further information and details the reader is recommended to consult papers I-V, which form the basis for this thesis.

## TOTAL ACTIVITY AND ISOZYME PATTERN OF LDH IN VARIOUS ORGANS (I, II, III).

The organs investigated during ontogeny are heart, liver, kidney, skeletal muscle and brain. The interest is focussed upon the gerbil, but comparisons are made with rat, mouse and rabbit (I, II). Both LDH activity and isozyme pattern of most of the organs change during the ontogeny, and differences between the species are revealed (I, II). In addition, the total activity and isozyme pattern of malate dehydrogenase (MDH, EC 1.1.1.37) have been investigated, reflecting the changes in activity of the tricarboxylic acid cycle during ontogeny.

It has been suggested that changes in LDH activity and isozyme pattern reflect metabolic and/or functional changes of an organ (Hyldgaard-Jensen, 1971; Greengard, 1971; Ogihara, 1975). It has been shown that hormones and environmental factors, such as oxygen pressure in cell cultures, influence the LDH pattern (Kaplan, 1965; Goodfriend et al., 1966; Hellung-Larsen & Andersen, 1969). In mice bearing Ehrlich ascites tumour the metabolic condition of the animals is influenced by the growing tumour. The tumour depletes the host serum of

its glucose (Nakamura & Hosoda, 1968), and the serum lactate concentration is elevated (Burgess & Sylvén, 1962) due to the high rate of glycolysis of the tumour (Racker, 1976). In order to investigate whether these changed serum concentrations of glucose and lactate affect the cellular metabolism, the total LDH activity of heart, liver, skeletal muscle and brain of mice bearing Ehrlich ascites tumour has been registered during tumour growth (III).

The method for measuring the total activity of LDH and MDH is described in (I). Registration of isozyme patterns of LDH and MDH has been performed according to the method described by Karlsson & Larsson (1971).

The isozymes of LDH, especially LDH 1 and LDH 5, are considered to play different roles in the cellular metabolism (Everse & Kaplan, 1975). LDH has been shown to form an inactive complex with pyruvate and $NAD^+$ at high pyruvate concentrations, and this complex-formation proceeds more readily with LDH 1 than with LDH 5 (Everse & Kaplan, 1973). The physiological significance of these findings is controversial (Vesell, 1975; Everse & Kaplan, 1975), and the function of LDH and its cooperation with $NAD^+$/NADH in the cellular metabolism will be discussed in the following text.

Liver

In the liver of the gerbil there is an increase in LDH activity during early neonatal development (I). However, the activity decreases during the ontogeny and in the adult liver the activity is at the same level as in the foetal liver of late gestational age. The ontogenetic changes in total MDH activity follow the same pattern as the LDH activity (I). Judging from the activities of LDH and MDH, the liver has equal capacity for anaerobic and aerobic carbohydrate metabolism.

However, the kind of metabolism is not solely determined by the enzyme activities. The amount of oxygen supply plays a great role in deciding whether the pyruvate is entering the tricarboxylic acid cycle or is converted to lactate. In an oxygenated cell the high $NAD^+/NADH$ quotient does not favour the conversion to lactate, and the liver is usually well oxygenated (Fischer, 1963; Pesch & Topper, 1963). LDH 5, which is the dominant isozyme in rodent livers (Jacobson et al., 1969; II) and has been shown to be the only one in gerbil liver (II), has been demonstrated to be associated with membrane structures and to be less active in this state (Hultin et al., 1972). Mammalian LDH 5 has also been shown to bind to blue dextran *in vitro* while LDH 1 does not (Nadal-Ginard & Markert, 1975). LDH 5 is released by NADH and it is assumed that NADH has the same function in the cell (Nadal-Ginard & Markert, 1975; Hultin, 1975). If this is true, the conversion of pyruvate to lactate is effectively prevented in an oxygenated liver cell, where reduced NAD is oxidized by the malate-aspartate shuttle (Lehninger, 1975).

The LDH 5 bound to blue dextran is also eluted by high concentrations of lactate in the presence of $NAD^+$ (Nadal-Ginard & Markert, 1975). Providing that lactate has the same ability *in vivo*, the activation of LDH 5 and the $NAD^+$ concentration of the oxygenated hepatocyte favours the conversion of lactate to pyruvate and subsequently to glucose when the liver takes part in the Cori cycle (Pesch & Topper, 1963; Cohen & Woods, 1976). In fact, when the blood lactate concentration is elevated, e.g. after intense work of the skeletal muscles, the liver is known to play a main role in the clearance of the lactate (Cohen & Woods, 1976).

In mice bearing Ehrlich ascites tumour the serum concentration of lactate is elevated (Burgess & Sylvén, 1962). This increase is caused by the high aerobic glycolysis of the tumour cells (Racker, 1976). The LDH activity of the liver of the tumour bearing mice is increased during tumour growth (III). This might be due to an increased synthesis of LDH

(Brinkworth & Masters, 1978), but may also be caused by the elevated substrate concentration. It is known that binding of the substrate to the enzyme protects the enzyme molecule against degradation and increases the half-life time (Grisolia, 1964). Moreover, the ability of lactate to release bound LDH 5 might make it possible to extract more enzyme during homogenization and consequently to register a higher enzyme activity.

Changes in an isozyme pattern of an organ do not necessarily mean that the isozyme pattern of the cells constituting the organ is changing. Altered proportions of the cell populations within the organ cause a changed isozyme pattern of the whole organ (Palmer & Karlsson, 1969). Our results indicate that the rabbit liver is an example of this (II). For a period of foetal life the liver is the main haemopoietic organ of mammals. Its importance decreases during late gestation and ceases during early neonatal development (Ackerman et al., 1961; Sorenson, 1963; Trávničková & Kraus, 1970; Jones, 1970). The fact that the percentage A-subunits of the rabbit liver increases from 30 to 60 during early neonatal development (II) and that mature red blood cells of the rabbit are dominated by B-subunits (Setchenska & Arnstein, 1978) indicates a shift in the cell populations of the liver, as a consequence of the cessation of the haemopoietic function. In the liver of gerbil, rat and mouse there is no corresponding transition of the LDH isozyme pattern (II). This is not to be expected, since both hepatocytes and red blood cells of these species are dominated by LDH 5 (Baur & Pattie, 1968; Jacobson et al., 1969; Shows et al., 1969; II). Consequently, changes in the haemopoietic cell population do not influence the isozyme pattern as clearly as in the rabbit liver.

Skeletal muscle

The LDH activity of gerbil skeletal muscle increases 5-fold during neonatal development (I). There is a concomitant increase in MDH activity, but it is not as great as for LDH (I). This increase in enzyme activity reflects the elevated metabolic activity of the muscle cells. The increase in LDH activity coincides with the increased locomotor activity of the animal after birth and the accompanying need for anaerobic glycolysis. Similar observations have been made concerning the pig (Hyldgaard-Jensen, 1971).

The LDH isozyme pattern does not change significantly during postnatal development (II), which might indicate that the muscle cells mature before birth. LDH 5 is the predominant isozyme in the skeletal muscle (II). This isozyme has a greater affinity for pyruvate than does LDH 1 (Pesce et al., 1967). Therefore it is a better catalyst of the reduction of the pyruvate accumulated in the skeletal muscle working under oxygen dificiency.

In mice bearing Ehrlich ascites tumour the LDH activity of skeletal muscle decreases during tumour growth (III). This lowered enzyme activity might be due to a progressively decreasing rate of protein synthesis (Lundholm et al., 1976), including that of LDH. However, a decreased LDH synthesis cannot account for the total decrease in LDH activity, as long as the half-life of muscle LDH of our mouse strain (NMRI) does not markedly differ from the one demonstrated for C57Bl/6J mice (Nadal-Ginard, 1978). A second reason for the lowered LDH activity may be an increased degradation of proteins, including LDH, in the skeletal muscle of the tumour bearing mice. An increased activity of proteolytic enzymes has been shown in patients with cancer (Lundholm et al., 1976; Theologides, 1979).

A third reason for the decreased LDH activity may be a decreased supply of glucose, due to the increasing glucose consumption of the growing tumour or a lowered capacity for glucose utilization similar to that observed in patients with cancer (Lundholm et al., 1978). A decreased glucose supply to the skeletal muscle may result in a decreasing metabolic activity, causing an energy deficiency induced deterioration of the cell membrane and a subsequent leakage of LDH molecules. This last suggestion is supported by the observation that the increased permeability of normal cell membranes to LDH is most probably a consequence of a decreased intracellular energy content (Sweetin & Thomson, 1973; Wilkinson & Robinson, 1974; V).

Heart

The total activity of gerbil heart LDH decreases precipitously after birth (I), whereas the decrease in the percentage of A-subunits is extended over a longer period of neonatal development (II). During the same period of ontogeny the MDH activity increases (I). The elevation of the percentage B-subunits and of MDH activity is in agreement with the known aerobic metabolism of the heart (Bing, 1965). The heart utilizes lactate in its metabolism (Bing, 1965; Drake, 1979) and the conversion to pyruvate, which subsequently enters the tricarboxylic acid cycle, is readily catalyzed by LDH 1 (Everse & Kaplan, 1975). It has been shown that the dog heart prefers lactate to glucose and free fatty acids and increases its uptake of lactate with increasing arterial lactate concentration (Drake, 1979).

The ability of the heart to increase its lactate utilization may cause the slight elevation of heart LDH activity of tumour bearing

mice. This elevated activity is observed during late tumour growth (III) when the blood lactate concentration is increased (Burgess & Sylvén, 1962). The substrate probably stabilizes the enzyme and prolongs the half-life (Grisolia, 1964). Since LDH 1 appears free in the cytoplasm (Hultin, 1975), the increased activity cannot be due to lactate mediated release of enzyme from membranes and a subsequent activation, which was suggested to be a possible reason for increased liver LDH activity of tumour bearing mice.

Kidney

The ontogenetic changes in total activity of LDH and MDH of the gerbil kidney show a great resemblance to those of the heart, with a rapid decrease in LDH activity immediately after partus and a pronounced increase in MDH activity during early neonatal development (I). This may indicate a greater capacity for aerobic metabolism. The idea is supported by the decrease in the percentage A-subunits (II),which may be a contribution from the developing cortex. The cortex is known to have a higher percentage B-subunits and a higher oxygen uptake than the rest of the kidney (Smith & Kissane, 1963; Kaplan, 1965; Bergelin & Karlsson, 1973). Moreover, the kidney utilizes lactate for both gluconeogenesis and oxidation in the tricarboxylic acid cycle (Cohen & Woods, 1976).

Brain

There are no significant changes in LDH activity of the gerbil brain during ontogeny, while the MDH activity increases during early neonatal development (I). On the other hand, the percentage A-subunits of LDH decreases after birth but the isozyme pattern of MDH does not

change during development (II).

The brain LDH activity of mice bearing Ehrlich ascites tumour is not affected by the growing tumour (III).

## LDH ACTIVITY IN PHYSIOLOGICAL FLUIDS (I, III, IV, V).

Elevated serum LDH activity has been registered during neonatal development of gerbil, rat and mouse (I). Whether this increase is caused by a greater leakage of enzyme from developing organs during the neonatal period, by the blood sampling method or by both is not clear. Blood was obtained from young animals by decapitation, since they do not bleed enough from the tail. Blood sampling by decapitation tends to increase the serum LDH activity (I), probably by rupturing of cells. In rabbit serum, where blood was collected from all animals by heart puncture, no corresponding elevation of enzyme activity in young animals was observed (I).

### Effect of tumour growth

It is well known that cell death causes increased LDH levels in physiological fluids (Wolfe & Williams, 1973). Changes in the isozyme pattern indicate the source of the enzyme, e.g. increased serum LDH 1 activity caused by myocardial infarction (Latner & Skillen, 1968; Wolfe & Williams, 1973). However, the registration of changes in the isozyme pattern is not always a reliable diagnostic tool, as cell death in liver or skeletal muscle both elevate serum LDH 5 activity (Latner & Skillen, 1963; Wolfe & Williams, 1973).

In gerbils bearing Ehrlich ascites tumour we have been able to identify the source of the increased LDH activity in cell free ascites fluid and serum (IV). The immunologically induced death

of tumour cells causes a release of LDH to the ascites fluid and a subsequent transition of the enzyme into serum. Both Ehrlich ascites tumour cells and gerbil serum contain only LDH 5 (IV). Since the tumour originates from mouse, the electrophoretic mobility of tumour LDH 5 is coincident with that of LDH 5 in mouse organs and different from the mobility of gerbil LDH 5 (II, IV). Thus electrophoretic separation of cell free ascites fluid and serum revealed that the activity of tumour derived LDH 5 in these body fluids increases with increasing tumour cell death and disappears after tumour regression (IV). This correlation between tumour cell death and elevated tumour derived LDH activity in body fluids is in agreement with the findings of other investigators (Pesce et al., 1977).

Also in mice bearing Ehrlich ascites tumour we have found increased levels of LDH activity in cell free ascites fluid and serum (III). The tumour cell death is not as large in the mice as in the gerbils (IV) and it cannot account for the total increase in extracellular LDH activity of the tumour bearing mice. The maximal theoretical release of LDH due to tumour cell death has been calculated and the value is exceeded by the LDH activity registered in the ascites fluid during tumour growth in mice (III). This result suggests that even viable tumour cells are leaking LDH into the ascites fluid. The idea is supported by the observations of other investigators. Firstly, malignant neoplasms have been found to cause increased LDH activity in host serum, whereas rapidly proliferating non-malignant tissue does not do this (Wróblewski, 1958). Secondly, Rous sarcoma cells release more LDH than cells infected with non-transforming Rous associated virus (Bissell et al., 1971). These observations suggest that leakage of LDH may be associated with a changed membrane function of transformed cells. In this respect it is notable that the high $Na^+$-$K^+$ ATPase

activity of Ehrlich ascites tumour cells most probably is caused by a lesion of the plasma membrane (Racker, 1976). An impairment of the cell membrane may result in an increased leakage of molecules into the extracellular fluid. Therefore it may be assumed that leakage of LDH from viable Ehrlich ascites tumour cells with an impaired membrane function is the main cause of the unexpectedly high LDH activity of the cell free ascites fluid (III).

Effect of partial hepatectomy

The effect of partial hepatectomy on plasma LDH activity of rats and mice has been investigated (V). Pronounced differences between the two species were revealed. Six hours after the operation the LDH 5 activity has increased 2-fold in rat plasma and 12-fold in mouse plasma. At 24 h the plasma LDH 5 level has returned to normal in the rat but still shows 5-fold elevation in the mouse (V). The maximum of rat plasma LDH activity at 6 h after partial hepatectomy coincides in time with the results of other investigators (Sekas & Cook, 1979), but the different maximum values may be a consequence of different blood sampling techniques, as discussed above in connection with newborn animals.

The ATP concentration is higher in rat liver than in mouse liver after partial hepatectomy (V) and our results seem to agree with the concept of LDH leakage being due to decreased cellular ATP concentration (Sweetin & Thomson, 1973; Wilkinson & Robinson, 1974), since LDH 5 is the dominant isozyme of both rat and mouse liver (II). However, the plasma clearance rate of LDH 5 has been shown to be significantly higher in the rat than in the mouse. Half lives of 0.5 and 2.1 h, respectively, are reported (Sinke et al., 1979;

Wachsmuth & Klingmüller, 1978). Furthermore, at least in the mouse, the clearance rate decreases after partial hepatectomy (Wachsmuth & Klingmüller, 1978). However, no change in this parameter can result in the 12-fold increase in LDH 5 activity observed in mouse plasma 6 h after partial hepatectomy (V). Thus it must be concluded that the cellular leakage of LDH 5 in the mouse increases after partial hepatectomy. Concerning the rat such a conclusion is not possible as even a moderate decrease in the plasma clearance rate can result in the observed increase in plasma LDH 5 activity. The decrease in mouse liver energy content, measured as energy charge levels (V), may suggest an effect of the partial hepatectomy on the cell membrane integrity of the liver remnant, resulting in an increased leakage of LDH.

In mouse liver LDH 5 is the dominant isozyme, whereas LDH 3 amounts to about 5 % (Karlsson & Larsson, 1971). The plasma LDH 3 activity found after partial hepatectomy is elevated more than could be expected from the percentage of LDH 3 in the liver (V). Thus the increased leakage of LDH in the partially hepatectomized mice cannot be resticted to the liver.

The normally low serum LDH activity (I) has been shown to increase to different degrees in tumour bearing and partially hepatectomized animals (III, IV, V). In the Ehrlich ascites tumour bearing gerbil, tumour cell death is the main cause of the 12-fold increase in serum LDH activity (IV). However, the leakage of LDH from viable tumour cells, as demonstrated for Ehrlich ascites tumour bearing mice (III), where the serum LDH activity increases 2.5 times, ought to occur also in tumour bearing gerbils, but will be masked by the greater release of LDH by dead or dying tumour cells.

In rats and mice subjected to partial hepatectomy, necrosis of the liver remnant might contribute to the increased plasma LDH activity (V). However, in our study no visible necrosis was observed. A temporary release of LDH from damaged cells at the operation site may also elevate the plasma LDH activity. This, in combination with a decreased plasma clearance rate, may be the cause of the transient increase in plasma LDH activity of partially hepatectomized rats. In mice the partial hepatectomy seems to have a more profound effect upon the animals, which is indicated by the greater elevation of plasma LDH activity, especially the unexpected rise in activity of LDH 3.

SUMMARY

1. Total activity and isozyme pattern of LDH and MDH changed to a varying degree in heart, liver, kidney, skeletal muscle, brain and serum during the ontogeny of the gerbil. Comparisons with the corresponding organs of developing rat, mouse and rabbit revealed differences between the species.

2. The gerbil liver exhibited only one LDH isozyme, LDH 5, througout the ontogeny.

3. In mice bearing Ehrlich ascites tumour the LDH activity of the liver increased markedly during tumour growth. A significant decrease of the LDH activity of the host skeletal muscle was observed during the same time period.

4. The LDH activity of serum and cell free ascites fluid of Ehrlich ascites tumour bearing mice was elevated during tumour growth. The increase in LDH activity of cell free ascites fluid was higher than that expected from tumour cell death.

5. In gerbils bearing Ehrlich ascites tumour a transient increase in LDH activity of serum and cell free ascites fluid was observed. The appearance and disappearance of tumour derived LDH in these body fluids are correlated to tumour cell death and tumour regression.

6. Rats and mice subjected to partial hepatectomy exhibited increased plasma LDH activities. The elevation of LDH activity persisted in mouse plasma 24 h after the operation, while it had returned to a normal level in rat plasma at this point of time.

ACKNOWLEDGEMENTS

This work has been carried out at the Department of Zoophysiology, University of Lund, Lund, Sweden. I wish to express my deep gratitude to my colleagues and friends at the department for encouraging discussions and inspiring collaboration.

Financial support has been received from the Royal Physiographical Society at Lund, the Hierta Retzius Foundation and the Foundation of Lars Hiertas Minne.

REFERENCES

Ackerman, G.A., Grasso, J.A. & Knouff, R.A. (1961) Lab. Invest. 10, 787-796.

Baur, E.W. & Pattie, D.L. (1968) Nature 218, 341-343.

Bergelin, I.S.S. & Karlsson, B.W. (1973) Int. J. Biochem. 4, 51-61.

Bing, R.J. (1965) Physiol. Rev. 45, 171-213.

Bissell, M.J., Rubin, H. & Hatié, C. (1971) Exptl. Cell Res. 68, 404-410.

Blanco, A. & Zinkham, W.H. (1963) Science 139, 601-602.

Brinkworth, R.I. & Masters, C.J. (1978) Int. J. Biochem. 9, 597-601.

Burgess, E.A. & Sylvén, B. (1962) Brit. J. Cancer 16, 298-305.

Cohen, R.D. & Woods, H.F. (1976) Clinical and Biochemical Aspects of Lactic Acidosis, pp. 10-39, Blackwell Scientific Publications, Oxford.

Drake, A.J. (1979) J. Physiol. (London) 289, 89P.

Everse, J. & Kaplan, N.O. (1973) Adv. Enzymol. 37, 61-133.

Everse, J. & Kaplan, N.O. (1975) Isozymes, Vol. 2 (Ed.: Markert, C.L.) pp. 29-43, Academic Press, Inc., New York, N.Y.

Fischer, A. (1963) The Liver. Morphology, Biochemistry, Physiology, Vol. 1 (Ed.: Rouiller, C.) pp. 329-378, Academic Press, New York & London.

Goodfriend, T.L., Sokol, D.M. & Kaplan, N.O. (1966) J. Mol. Biol. 15, 18-31.

Greengard, O. (1971) Essays in Biochemistry, Vol. 7 (Eds.: Campbell, P.N. & Dickens, F.) pp. 159-205, Academic Press, Inc., New York, N.Y.

Grisolia, S. (1964) Physiol. Rev. 44, 657-712.

Hellung-Larsen, P. & Andersen, V. (1969) Exptl. Cell Res. 54, 201-204.

Hultin, H.O. (1975) Isozymes, Vol. 2 (Ed.: Markert, C.L.) pp. 69-85, Academic Press, Inc., New York, N.Y.

Hultin, H.O., Ehmann, J.D. & Melnick, R.L. (1972) J. Food Sci. 37, 269-273.

Hyldgaard-Jensen, J.F. (1971) Lactate dehydrogenase in pigs. Studies of lactate dehydrogenase isoenzymes in blood and organs, Thesis, Munksgaard, Copenhagen.

Jacobson, K.B., Murphy, J.B. & Dunaway, P.B. (1969) Comp. Biochem. Physiol. 28, 1135-1144.

Jones, R.O. (1970) J. Anat. 107, 301-314.

Karlsson, B.W. & Larsson, G.B. (1971) Comp. Biochem. Physiol. 40B, 93-108.

Kaplan, N.O. (1965) J. Cell. Comp. Physiol. 66, 1-10.

Latner, A.L. & Skillen, A.W. (1968) Isoenzymes in Biology and Medicine, pp. 146-154, Academic Press, Inc., London.

Lehninger, A.L. (1975) Biochemistry, 2nd ed., pp. 533-536, Worth Publishers, Inc., New York, N.Y.

Lindahl, R. & Mayeda, K. (1972) Comp. Biochem. Physiol. 43B, 425-433.

Lundholm, K., Bylund, A.-C., Holm, J. & Scherstén, T. (1976) Europ. J. Cancer 12, 465-473.

Lundholm, K., Holm. G. & Scherstén, T. (1978) Cancer Res. 38, 4665-4670.

Markert, C.L. (1968) Ann. N.Y. Acad. Sci. 151, 14-40.

Masters, C.J. & Holmes, R.S. (1975) Frontiers of Biology, Vol. 42. Haemoglobin, Isoenzymes and Tissue Differentiation (Eds.: Neuberger, A. & Tatum, E.L.) pp. 156-165, North-Holland Publishing Company, Amsterdam.

McKusick, V.A. & Ruddle, F.H. (1977) Science 196, 390-405.

Nadal-Ginard, B. & Markert, C.L. (1975) Isozymes, Vol. 2 (Ed.: Markert, C.L.) pp. 45-67, Academic Press, Inc., New York, N.Y.

Nadal-Ginard, B. (1978) J. Biol. Chem. 253, 170-177.

Nakamura, W. & Hosoda, S. (1968) Biochim. Biophys. Acta 158, 212-218.

Ogihara, M. (1975) Isozymes, Vol. 2 (Ed.: Markert, C.L.) pp. 157-170, Academic Press, Inc., New York, N.Y.

Palmer, L.S. & Karlsson, B.W. (1969) Can. J. Biochem. 47, 1171-1178.

Pesce, A., Fondy, T.P., Stolzenbach, F., Castillo, F. & Kaplan, N.O. (1967) J. Biol. Chem. 242, 2151-2167.

Pesce, A.J., Bubel, H.C., DiPersio, L. & Michael, J.G. (1977) Cancer Res. 37, 1998-2003.

Pesch, L.A. & Topper, Y.J. (1963) The liver. Morphology, BIochemistry, Physiology, Vol. 1 (Ed.: Rouiller, C.) pp. 605-633, Academic Press, New York & London.

Racker, E. (1976) J. Cell. Physiol. 89, 697-700.

Schwentker, W. (1975) The Gerbil. An annotated bibliography of the gerbil as an experimental animal in medical research. Tumblebrook Farm, West Brookfield, Ma.

Sekas, G. & Cook, R.T. (1979) Br. J. Exp. Path. 60, 447-452.

Setchenska, M.S. & Arnstein, H.R.V. (1978) Biochem. J. 170, 193-201.

Shows, T.B., Massaro, E.J. & Ruddle, F.H. (1969) Biochem. Genet. 3, 525-536.

Sinke, J., Bouma, J.M.W., Kooistra, T. & Gruber, M. (1979) Biochem. J. 180, 1-9.

Smith, C.H. & Kissane, J.M. (1963) Develop. Biol. 8, 151-164.

Sorenson, G.D. (1963) Ann. N.Y. Acad. Sci. 11, 45-69.

Sweetin, J.C. & Thomson, W.H.S. (1973) Clin. Chim. Acta 48, 403-411.

Theologides, A. (1979) Cancer 43, 2004-2012.

Trávníčková, E. & Kraus, R. (1970) Physiol. Bohemoslov. 19, 55-66.

Vesell, E.S. (1975) Isozymes, Vol. 2 (Ed.: Markert, C.L.) pp. 1-28, Academic Press, Inc., New York, N.Y.

Wachsmuth, E.D. & Klingmüller, D. (1978) J. Reticuloendoth. Soc. 24, 227-241.

Wilkinson, J.H. & Robinson, J.M. (1974) Clin. Chem. 20, 1331-1336.

Wolfe, P.L. & Williams, D. (1973) Practical Clinical Enzymology, Wiley-Interscience (John Wiley & Sons), New York, N.Y.

Wróblewski, F. (1958) Ann. N.Y. Acad. Sci. 75, 322-338.

AV-centralen i L 80